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Ultrastructural Study of the Tetrathyridium of *Mesocestoides corti* Hoeppli, 1925 (Cestoda): Pool of Germinative Cells and Suckers [1]

by

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With 11 figures

Abstract

The study of normal and regenerating tetrathyridia shows that 2 tissues of this larva are capable to proliferate by mitosis: a) the apical massif (described by Hess 1980), and b) the pool of germinative cells. The latter occur as dividing forms (light germinative cells) and as migrating forms (dark germinative cells). Dark germinative cells develop into parenchymal muscle cells.

The suckers of the tetrathyridia are composed of five different muscle systems, an interstitial syncytium, glycogen-storing sucker cells, and neurons with different functions. During morphogenesis, the sucker blastema is isolated by fibroblasts from the surrounding tissues. The origin of the blastema cells is discussed.

INTRODUCTION

Following the papers dealing with the tegument and the parenchyma of the tetrathyridium of *Mesocestoides corti*, (Hess & Guggenheim 1977; Hess 1980), the structure of the germinative cells and of the suckers will be described here. According to our knowledge, the structure of the cyclophyllidean sucker has never been analysed exhaustively by means of electron microscopy. The pool of germinative cells, on the other hand, plays an important role in regeneration and asexual multiplication of the tetrathyridia.

[1] Part of the author's thesis.

MATERIALS AND METHODS

Tetrathyridia of *Mesocestoides corti* originally isolated by SPECHT & VOGE (1965) and cultivated in our laboratory in NMRI-mice were used in this study. Tissue descriptions were made on larvae with four suckers having terminated regeneration. Differentiation of the tissues was studied in tetrathyridia at various stages of asexual multiplication. Transmission electron microscope preparation techniques (glutaraldehyde/OsO_4-fixation were those described by HESS & GUGGENHEIM (1977). Ultra-thin sections were studied on a Philips 201 EM.

RESULTS AND CONCLUSIONS

The pool of germinative cells

RESULTS

Two types of small, free and undifferentiated cells occur in the parenchyma of the tetrathyridia. We call them dark and light germinative cells [1].

The light germinative cells which are of oval shape (5-6 μm ∅) are scattered throughout the parenchyma. They contain free ribosomes, few or no RER and some small, oval mitochondria. They divide by mitosis (fig. 1). On semifine sections stained with toluidine-blue they are hardly visible.

The dark germinative cells which are spindle-shaped (up to 10 μm long) occur also in the parenchyma. They are however more numerous near the parenchymal muscle layer, where they are seen to transform into myoblasts (fig. 2). They possess long pseudopods. Their cytoplasm has numerous free ribosomes, few or no RER and microtubules. Their mitochondria are longish or of oval shape. These cells do not divide. On semifine sections, the dark germinative cells strike by their allochomasy produced with toluidine-blue.

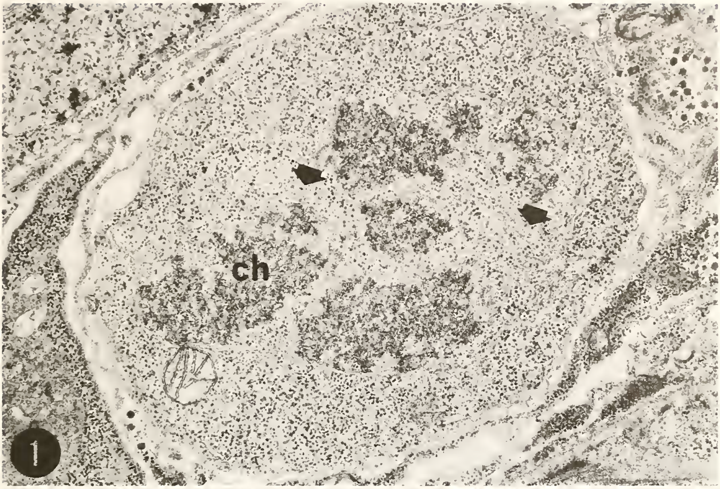
[1] Germinative cell: term created by WISNIEWSKI (1930) and used by WIKGREN & GUSTAFSSON (1967) and von BONSDORFF *et al.* (1971) to designate undifferentiated proliferating cells of *D. dentriticum.*

FIG. 1.

Tetrathyridium of *Mesocestoides corti.* Light germinative cell during mitosis. ch: chromosome; arrows: spindle tubules, (19 750 x).

FIG. 2.

Tetrathyridium of *Mesocestoides corti.* Transversal section of a parenchymal muscle bundle. mc: myocyte with muscle fibres (fb); dg: dark germinative cell with pseudopod; ne: neuron with dense core vesicles and axon (11 400 x). Inset: cytoplasmic process of germinative cell transforming into myoblast. In the cytoplasmic extensions (arrows) originating from the pseudopod (p) contractile muscle elements will be synthesized. (27 000 x).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When dark germinative cells transform into myoblasts, the pseudopods become permanent cytoplasmic processes. Cellular outgrowths originating from the distal parts of the cytoplasmic processes differentiate into muscle fibres following the pattern of differentiation of the subtegumental muscles (HESS 1980).

DISCUSSION

Cells which are capable of mitotic divisions habe been described from cestodes by several authors. WISNIEWSKI (1930), WIKGREN & GUSTAFSSON (1967, 1971), WIKGREN & KNUTS (1970), von BONSDORFF *et al.* (1971) called them "germinative cells" while other authors called them "free cells", "cellules souches" (PRENANT 1922) or "Plastinzellen" (VOGEL 1930). Comparable cells of turbellarian are the "neoblasts" or "beta-cells" (HAY & COWARD 1975).

Two theories about dividing cells of cestodes have been established. WISNIEWSKI (1930) and DOUGLAS (1961) believe that two types of dividing cells occur: i.e. a) "gonial cells" or "germinative Zellen" which are a stock of embryonic reproductive cells, and b) "somatic germinative cells" which are the embryonic stock of the soma. The other theory, supported by VOGEL (1930), RIBYCKA (1966), von BONSDORFF *et al.* (1971) and others proposes that only one type of embryonic cells occurs, called "Plastinzellen" or "germinative cells", yet present in the oncosphere.

In the tetrathyridia two different pools of dividing cells have been observed: the apical massif, which differenciates during asexual multiplication into tegumental syncytium, tegumental muscles and glycogen-storing parenchyma cells (HESS 1980), and the germinative cells which transform into parenchymal muscle cells. There is no doubt that the parenchymal muscles derive from germinative cells but it has not been possible to determine if the cells of the excretory and osmoregulatory system, calcareous corpuscule cells, or neurons develop also from germinative cells. Interactions between the apical massif and the pool of germinative cells have not been observed but cannot be excluded.

According to our knowledge, light and dark germinative cells represent two physiological stages of the same type of cells. The light germinative cells are proliferating germinative cells, while the dark cells are germinative cells during migration or beginning differentiation. WIKGREN & GUSTAFSSON (1971) and von BONSDORFF *et al.* (1971) do not distinguish between light and dark germinative cells.

FIG. 3.

Tetrathyridium of *Mesocestoides corti*. Sagittal section of the sucker. sc: superficial cytoplasm of the tegumental syncytium; t: transversal upper muscles; l: longitudinal upper muscles; p: perpendicular muscles; o: transversal lower muscles; mc: perinuclear cell body of a myocyte; is: perinuclear part of the interstitial syncytium; gc: lobes of glycogen-storing sucker cells; f: cytoplasmic processes of fibrocytes; arrows: fibrous sheet. (4 700 x).

FIG. 4.

Tetrathyridium of *Mesocestoides corti*. Transversal section of perpendicular muscle fibres (mf) of the sucker; t: subsurface tubular system; gc: glycogen-storing sucker cell; arrows: cytoplasmic bridges. (53 000 x).


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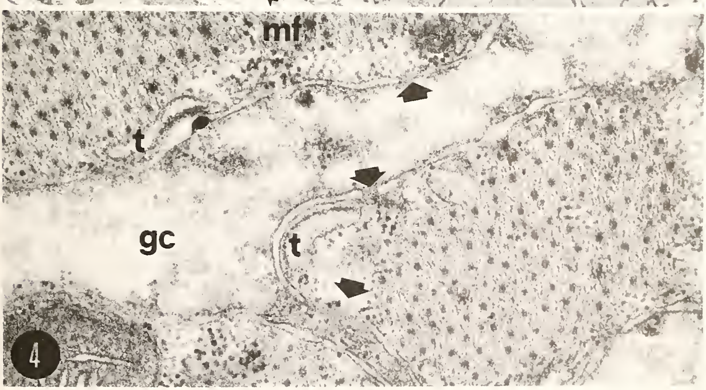
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The suckers

Results

The four adhesive organs of the tetrathyridium are cupshaped suckers situated on the top of the scolex. They lie immediately below the superficial cytoplasm of the tegumental syncytium (fig. 3). They are composed of myocytes, glycogen-storing sucker cells, an interstitial syncytium and neurons.

Each sucker is separated from the surrounding tissues by a fibrous sheet, thus it does not have any cellular continuity with the tegument or with the parenchyma. The fibrous sheet is the attachment layer for the internal sucker muscles. At its convex face, it is covered by one or several thin cytoplasmic layers which are processes of degenerated fibrocytes (Fig. 5).

Muscle cells

The major part of the sucker is formed by muscle tissue. For a better comprehension of the muscle fibres arrangement, one has to imagine the cup shaped sucker extended as a disc. We distinguish the following muscle layer (Fig. 3):

1. The transversal upper muscle system, whose fibres are parallel to the circular subtegumental muscle fibres. These are the most superficial muscles.
2. The longitudinal upper muscle system, whose fibres are parallel to the longitudinal subtegumental muscle fibres. They lie below the transversal upper muscles.
3. The perpendicular muscle system whose fibres are perpendicular to the subtegumental muscle fibres and to the other muscle fibres of the sucker. They cross the sucker reaching from the upper to the lower face of the fibrous sheet.
4. The transversal lower muscle system whose fibres are parallel to the circular subtegumental muscle fibres.
5. The longitudinal lower muscle system, whose fibres are parallel to the longitudinal subtegumental fibres.

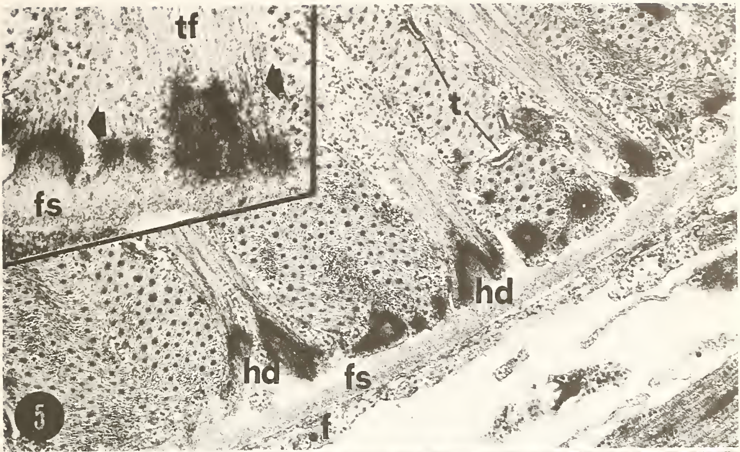
The strongest fibres are those of the transversal lower and the perpendicular muscle systems (up to 2 μm diameter). The fibres of the other muscles have about 1 μm diameter except for the longitudinal lower fibres which are less developed and of smaller diameter.

Fig. 5.

Tetrathyridium of *Mesocestoides corti*. Sagittal section of the sucker. f: cytoplasmic processes of fibrocytes covering the fibrous sheet (fs); hd: hemi-desmosomes; t: subsurface tubular system. (31 200 x). Inset: tonofibrillae (tf) attaching to the hemi-desmosome; (70 000 x).

Fig. 6.

Tetrathyridium of *Mesocestoides corti*. Transverse section of the sucker. pm: fibres of perpendicular muscles; gc: glycogen-storing sucker cell; is: interstitial syncytium with nuclei (N). (12 500 x).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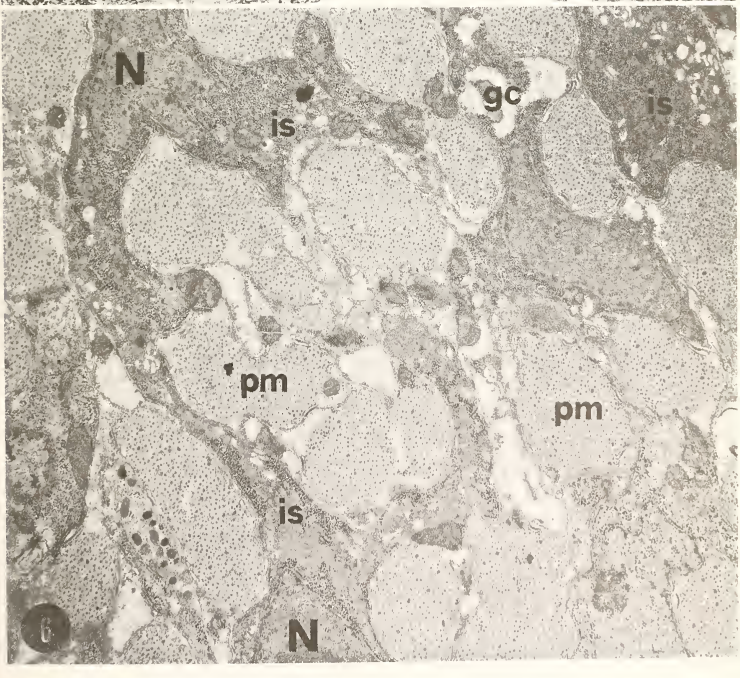
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Each myocyte of the sucker muscles has several fibres. Cytoplasmic bridges between muscle fibres and glycogen-storing sucker cells are frequent (Fig. 4). The perinuclear cell bodies of the myocytes are predominantly situated in the lower part of the sucker. The fine structure of the sucker myocytes is comparable to the parenchymal and tegumental muscles (Hess 1980). Two particular features which do not occur either in the parenchymal or in the tegumental muscles have however been noticed in the sucker muscles: a) a strongly developed sub-surface tubular system (Figs. 4, 5), and b) the attachment mode of the muscle fibres to the fibrous sheet by means of hemi-desmosomes (Fig. 5).

Interstitial syncytium

At the concave face of the sucker, an important syncytial cell mass occurs between the muscle fibres. The fine structure of this interstitial syncytium is similar to young tegumental cells (Hess 1980). The hyaloplasm is of high electron-density and contains free ribosomes, RER, mitochondria and Golgi apparatus (Figs. 3, 6).

Glycogen-storing sucker cells

Voluminous cells which contain predominantly alpha-glycogen occupy the space between the muscle fibres and the interstitial syncytium (Figs. 3, 4, 5, 6). Alpha-glycogen is stored in large lobes in which are also found mitochondria and lipid droplets. Ribosome containing hyaloplasm is predominantly concentrated in the perinuclear zone. The glycogen-storing cells are connected to the muscle cells by cytoplasmic bridges as described above (Fig. 4).

Nervous tissue

Each sucker is innervated by at least two large nerves which have their origin in the anterior ganglion complex and ramify in the sucker (Hart 1967). In the upper part of the sucker, specialized nervous endings are frequently observed which contain a large number of electron-dense vesicles. These vesicles give a positive reaction with the staining methods of Mann-Dominici and Gomori (light microscopy) which are considered to be specific for neurosecretions.

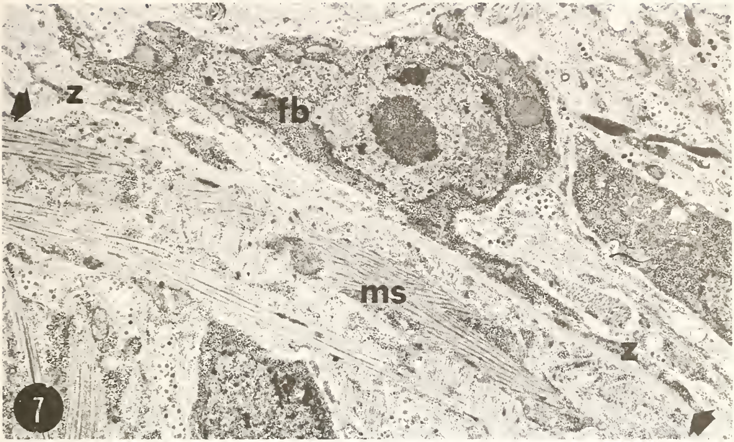
Sensory nervous processes as described by Hess & Guggenheim (1977) occur in the superficial cytoplasm covering the suckers. The dendrites pierce the fibrous layer of the tegument and project into the sucker tissue. It is not known whether the pericaryons of these neurons lie in the sucker or in the cephalic ganglions.

Fig. 7.

Tetrathyridium of *Mesocestoides corti*. Fibrocyte (fb) having synthesized the fibrous sheet (arrows); z: cytoplasmic processes; ms: sucker muscles, (11 600 x).

Fig. 8.

Tetrathyridium of *Mesocestoides corti*. Regenerating sucker.
tc: perinuclear cell bodies of the tegumental syncytium having detached from the superficial cytoplasm (sc) to form the interstitial syncytium; arrow: fibrous sheet. (11 000 x).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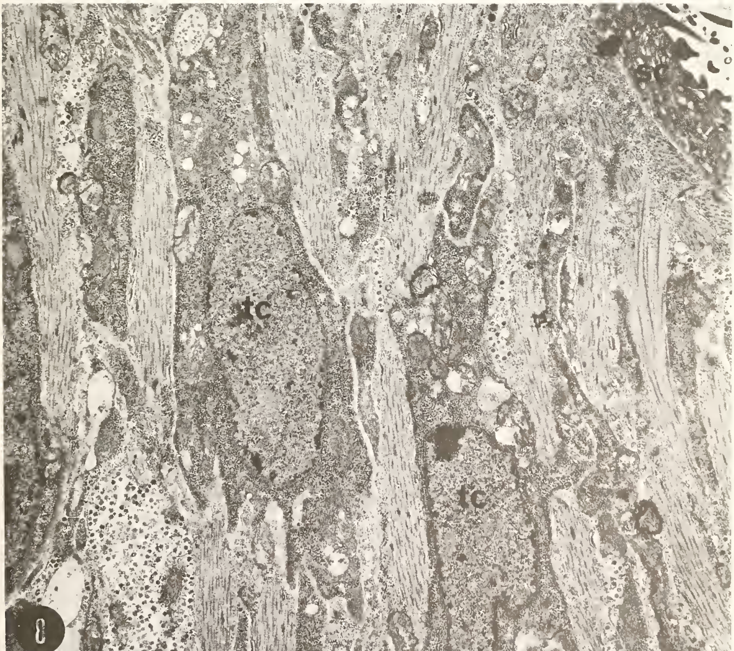
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Morphogenesis of the suckers during asexual multiplication

The new suckers develop from the medio-lateral parts of the dividing scolex (Hess 1980). First, fibroblasts isolate each sucker blastema from the surrounding tissue. These fibroblasts have ribosome-and RER-rich cytoplasm which contains large cysternae. They synthesize the fibrous sheet towards the sucker blastema (Fig. 7). Near the tegument, the fibrous sheet fuses with the fibrous layer of the tegument. After formation of the fibrous sheet, the volume of the fibroblasts decreases and the RER disappears. The cells seem to degenerate but remain attached to the fibrous sheet as thin cytoplasmic layers (Figs. 3, 5).

The blastema isolated by the fibrous sheet contains five different types of cells:

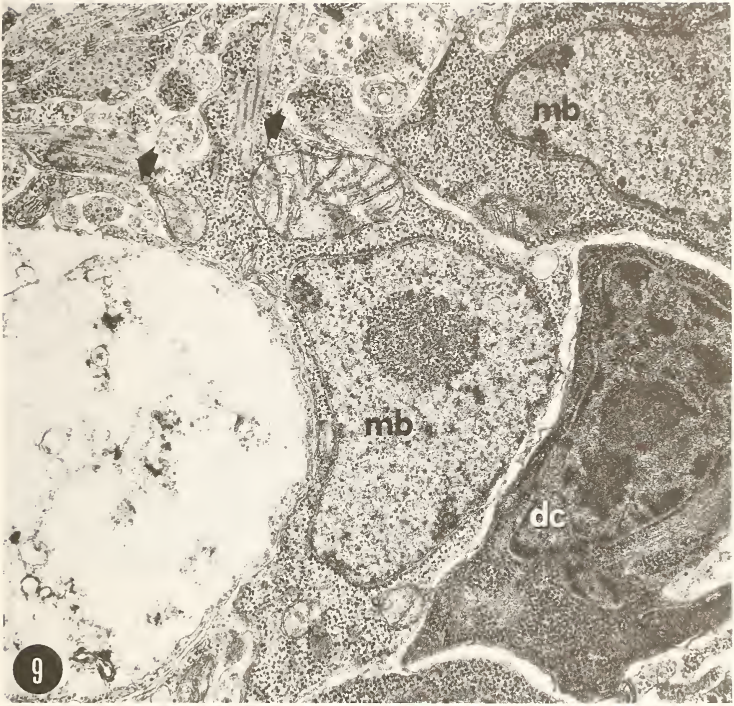
1. Near the superficial cytoplasm of the tegument, longish cells, which are arranged perpendicularly to the tegument, fuse to form the interstitial syncytium (Fig. 8). These cells are perinuclear cell bodies of the tegumental syncytium which separate from the superficial cytoplasm, thus loosing any cytoplasmic continuity with the latter. Their fine structure is similar to young tegumental cell bodies: dense ribosome-rich cytoplasm, surface cysternae-forming RER and Golgi apparatus.
2. Undifferentiated cells with long, pseudopod-like expansions occur below the interstitial syncytium. Their cytoplasm is electron-dense and contains RER, free ribosomes and some small mitochondria (Fig. 9). They are identical or comparable to dark germinative cells.
3. The myoblasts of the regenerating suckers have long cytoplasmic processes. The electron-density of their cytoplasm varies from dense to lucid. It is rich in free and RER-bound ribosomes and endoplasmatic cisternae (Fig. 9). The synthesis of the myofilaments in the cytoplasmic processes follows the same pattern as described for the subtegumental myoblasts (Hess 1980).
4. Electron-dense undifferentiated cells accumulate alpha-glycogen (Fig. 10). They transform into large glycogen-storing cells. The fully developed glycogen-storing cells have lost the original electron-density of the hyaloplasm and possess large lobes filled with alpha-glycogen.
5. Some cells of the sucker anlage having some RER produce electron-dense granules in zones of SER (Fig. 11). These cells are considered to be precursors of neurons, but their functional modality (motor, sensory, neurosecretory) cannot be determined.

Fig. 9.

Tetrathyridium of *Mesocestoides corti*. Regenerating sucker.
dc: dark cell similar to dark germinative cell;
mb: myoblasts with differentiating muscle fibres (arrows). (16 200 x).

Fig. 10.

Tetrathyridium of *Mesocestoides corti*. Regenerating sucker. Dark cell (dc) accumulating alpha glycogen (ag) in peripheral lobes. (25 100 x).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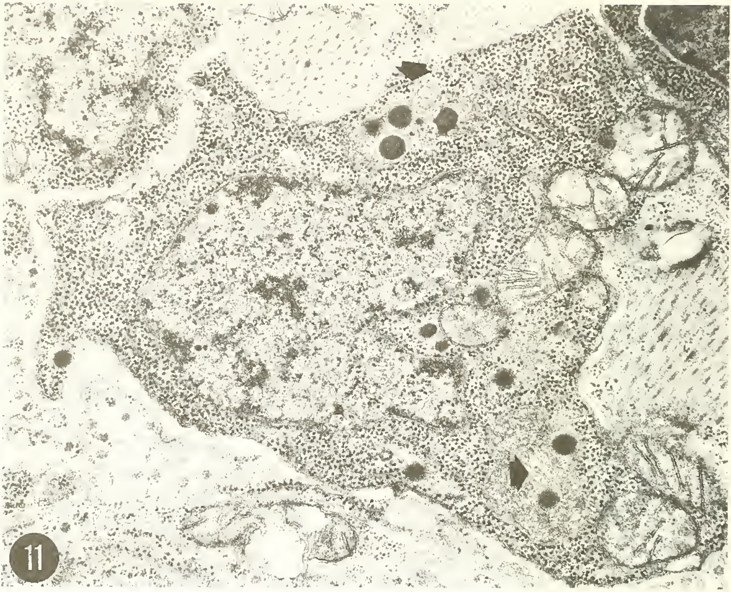

FIG. 11.

Tetrathyridium of *Mesocestoides corti*. Sucker blastema. Neuroblast with dense core vesicles (arrows). (26 800 x).

DISCUSSION

The suckers of the tetrathyridium are highly specialized organs. They are predominantly composed of cell types which are homologous to cells of the tegument and the parenchyma.

The interstitial syncytium is homologous to the tegumental syncytium, as demonstrated by its morphogenesis from the latter during asexual multiplication. It represents an isolated part of the tegumental syncytium whose function is not clear. It is improbable that it has a trophic function comparable to the tegumental cell bodies, as it lacks any cytoplasmic continuity with the superficial cytoplasm of the tegument. This hypothesis is confirmed by the fact that the typical vesicles associated with the Golgi apparatus of the perinuclear cell bodies of the tegumental syncytium (HESS 1980) are absent in the interstitial syncytium.

The sucker muscles, except for the perpendicular muscles, are homologous to the subtegumental muscles. This is evident from their arrangement and the fact that no tegumental muscles appear between the suckers and the superficial cytoplasm of the

tegument. The perpendicular muscles are probably homologous to the longitudinal parenchymal muscle system. The myocytes of the suckers are distinguished by two features from subtegumental and parenchymal muscle cells. The sucker muscle fibres attach by hemi-desmosomes to the fibrous sheet, while tegumental and parenchymal muscles are immersed into the fibrous layer. The first type of attachment provides without doubt a better attachment of the muscle fibres. This adaptation to increased stress is easily understood if one knows that the suckers of the tetrathyridia are not simply hold-fasts, but that they are also used to lacerate the tissues during the migrations of the larva in the host. The second difference between the sucker muscles and the other muscles of the tetrathyridia is the presence of a subsurface tubular system which occurs only in the first. It is possible that these tubules are homologous to the T-system of the striated muscle, but it is not understood why this transport-system would be restricted to the sucker muscles.

The glycogen-storing sucker cells have the same fine structure as the parenchymal glycogen-storing cells; they are considered to be homologous to the latter. The function of glycogen-storing cells is a trophical one. They synthesize and store alpha-glycogen. This compound can be degraded to beta-glycogen and transferred directly into the muscle fibres by the intercellular bridges. Cytoplasmic bridges between glycogen-storing cells and myocytes are also observed in the parenchyma (Hess 1980).

The first step of the morphogenesis of the suckers during asexual multiplication takes place when fibroblasts isolate the sucker blastema from the surrounding tissues. They synthesize the fibrous layer and subsequently degenerate. Their origin is not clear and we do not know what kind of stimulus activates them.

The dark undifferentiated cells which are found in the regenerating sucker may be considered to be the precursors of the glycogen-storing sucker cells and the sucker muscles. They either belong to the pool of germinative cells or derive from the apical massif. As demonstrated recently, both subtegumental muscles and parenchymal glycogen-storing cells originate from the apical massif (Hess 1980). If the sucker muscles and the glycogen-storing sucker cells are homologous to the subtegumental muscles and the glycogen-storing parenchyma cells, one would suppose an identical origin of all these tissues, i.e. the apical massif. This could however not be demonstrated unequivocally as the presence or absence of germinative cells in the sucker anlage cannot be determined. This problem is related to the question of whether there exist interactions between the pool of germinative cells and the apical massif or not.

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